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Certificate of Analysis

pUSE *raf-1* (wild type) 6.5kb

Catalog # 21-111
Lot # 16607

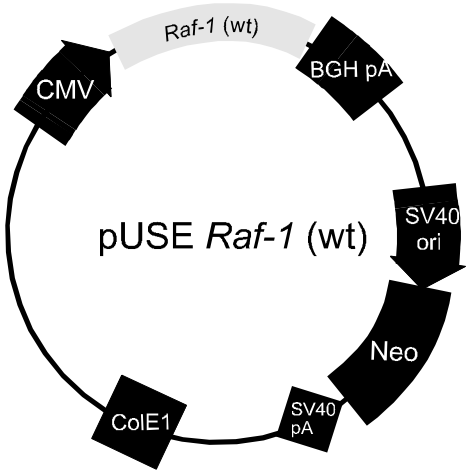
Product Description: Transfection grade eukaryotic expression vector containing the human *Raf-1* (wild type) cDNA under control of the CMV promoter as illustrated.

The cDNA was inserted as a BamHI fragment into the BamHI site of the pUSE(-) multiple cloning site (Catalog # 21-101).

Formulation: **50mg** affinity purified DNA eluted, packaged and lyophilized in 10mM Tris-HCl, pH 7.4, 1mM EDTA aseptically. Reconstitute in100µl sterile water to 0.5mg/ml.

Storage and Stability: Store lyophilized and reconstituted DNA at –20ºC. Reconstituted DNA is stable at –20ºC for 6 months. Lyophilized DNA is stable at -20ºC for 1 year from date of shipment.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS



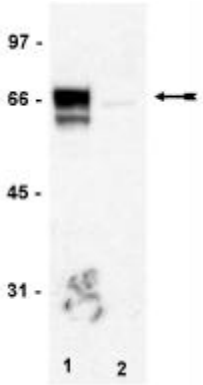
This cDNA construct is designed and intended for mammalian cell transfection only. It is **not** intended for propagation in bacteria and does **not** encode ampicillin resistance.

Quality Control Testing

Diagnostic DNA Digest: This lot of DNA was cut with Hind III, which generated the expected fragments of 1.6 and 4.9 kb.

Purity: The OD_{260}/OD_{280} for this lot of pUSE *Raf-1* is 1.85.

Transient Transfection Assay: Expression of Raf-1 was detected by immunoblotting of pUSE *raf-1* (wild type) transfected Cos-1 cells with 1µg/ml anti-Human Raf-1 CT (Catalog # 06-253)..



Immunoblot Analysis

RIPA lysates of transiently transfected Cos-1 cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Human Raf1-CT (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Lane 1, pUSE *Raf-1* (wild type) transfected cells; Lane 2, untransfected control. Arrow indicates Raf-1.

Background: The activation of Raf-1 is essential for the transmission of many proliferative, developmental and oncogenic signals within the cell. This serine-threonine protein kinase phosphorylates and activates the mitogen-activated protein (MAP) kinase kinase, aiding in the perpetuation of the signal through the MAP kinase pathway. Normal activation of Raf-1 is dependent on the Ras proto-oncogene product. Mutations preventing the Raf-1:Ras interaction have been identified.

References:

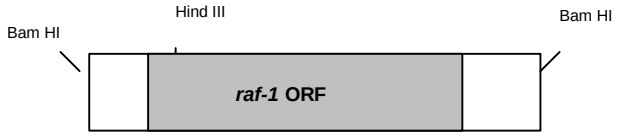
Culter, R. and Morrison, D., EMBO **16:** 1953-1960, 1997.
Leevers, S., *et al.*, Nature **369**: 411-412, 1994.
Dent, P., *et al.*, Science **268:** 1902-1905, 1995.

Plasmid Information:

Multiple Cloning Site of pUSE (-) [5'? 3']:

Nhe I
Pme I
Apa I
Xba I
Xho I
Not I
BstX I
EcoR V
EcoR I
BstX I
BamH I
Asp718 I
Kpn I
Hind III
Afl II
PmeI

Schematic Map of *raf-1* cDNA :



Restriction Endonuclease Map by Position: **pUSE(-)** **Catalog # 21-101**

(Frequency < 3; Recognition sites >5 bp)
Multiple cloning site: Nhe I @ 895 nt to Pme I @ 1006 nt.

12 Bgl II
12 NcrI
31 Rrh4273I
32 Sal I
55 StySJ
80 AlwN I
161 MfeI
161 Mun I
180 Bpu10 I
208 Nru I
228 Afl III
228 Mlu I
249 Spe I
333 BsoJI
340 Bgl I
422 Taq II'
455 BsoJI
462 Bgl I
484 Nde I
526 BsoJI
533 Bgl I
537 BfiI
549 BmrI
590 BsaA I
590 Eco105 I
590 SnaB I
610 Bsp19I
610 Dsa I
610 Nco I
615 Msl I
626 AsuHPI
626 Hph I
684 Alw26 I
684 BsmAI
816 EcoICR I
818 Sac I
818 SstI
876 Alw26 I
876 Bsa I
876 BsmAI
876 Eco31I
895 Nhe I
899 Ace II
904 Dra I
904 Pme I
909 Bsp120 I
910 EcoO109 I
913 Apa I
913 PpeI
913 Pss I
915 Xba I
920 BsuMI
920 Sex I
921 AquI
921 Ava I
921 Ccr I
921 Eco88I
921 Xho I
922 Ccr I
923 Sci I
925 Nli387/7 I
926 Sex I
927 CciNI
927 Eco52 I
927 Not I
927 Xma III
940 BstX I
943 M.SmaDam
946 EcoR V
953 Pst I
954 EcoR I
954 HaII
954 RsrI
954 SsoI
966 BstX I
971 Spe I
977 AccEBI
977 BamH I
977 BnaI
985 EcoICR I
987 Sac I
987 SstI
989 Acc65 I
989 Asp718 I
993 Kpn I
995 Bbr I
995 EcoVIII
995 Hind III
998 Afl II
998 Bfr I
998 Bst98 I
1006 Dra I
1006 Pme I
1013 BmeTI
1014 Bcl I
1036 EcoR124 I
1216 Bbs I
1273 BavI
1273 Pvu II
1422 NgoM I
1424 Nae I
1424 SauLPI
1524 AdeI
1527 AsuHPI
1527 BsaA I
1527 Hph I
1530 Dra III
1533 AdeI
1574 Drd I
1584 Bsa XI
1586 UbaD I
1683 Ace III
1725 Xmn I
1799 BstAPI
1800 ApaB I
1800 Ppu10 I
1802 BfrB I
1804 Nsi I
1821 SexA I
1871 BstAPI
1872 ApaB I
1872 Ppu10 I
1874 BfrB I
1876 Nsi I
1931 BfiI
1943 BmrI
1961 Bsp19I
1961 Dsa I
1961 Nco I
2050 BseR I
2052 Aat I
2053 Bln I
2053 Stu I
2054 Aat I
2054 Avr II
2054 Bln I
2075 AquI
2075 Ava I
2075 Cfr9I
2075 Eco88I
2075 PspAI
2075 Xma I
2077 CfrJ4I
2077 Sma I
2079 Nli387/7 I
2104 BmeTI
2105 Bcl I
2106 Alw26 I
2106 BsmAI
2123 BsaB I
2151 BspM I
2168 Taq II'
2170 Eco52 I
2170 Xma III
2202 BmrI
2213 BfiI
2263 Kas I
2263 Nun II
2264 Nar I
2264 Nun II
2265 Ehe I
2267 Bbe I
2291 Drd I
2295 BsaW I
2317 Pst I
2343 Msp20 I
2346 Bal I
2346 MscI
2349 Msp20 I
2366 Fsp I
2370 BavI
2370 Pvu II
2382 Tth111 I
2410 BspKT5I
2410 Eco57 I
2442 AsuHPI
2442 Hph I
2489 BseMI
2497 BsrD I
2532 BspM I
2545 EcoR124 II
2568 BsaA I
2608 Ear I
2608 Sap I
2660 BscE I
2660 BsePI
2661 BssH II
2666 BscE I
2666 BsePI
2696 Bsp19I
2696 Dsa I
2696 Nco I
2701 Msl I
2760 Taq II'
2764 NgoM I
2766 Nae I
2766 SauLPI
2780 Cpo I
2780 Csp I
2818 Ear I
2818 Sap I
2842 BspKT5I
2842 Eco57 I
2856 BssS I
2945 Asu II
2946 Asu II
2946 Bst B1
2946 Cbi I
2946 Csp45 I
2946 Sfu I
2982 BspM I
2982 BssS I
3047 Bpm I
3047 NgoM I
3049 Nae I
3049 SauLPI
3077 EcoD XXI
3104 Bpm I
3183 CfrA I
3184 BsaM I
3236 Bst1107 I
3240 Rrh4273I
3241 Sal I
3439 BavI
3439 Pvu II
3478 Bsa XI
3495 StyLT III
3499 Ear I
3499 Sap I
3567 Ace III
3615 Afl III
3615 BspLU11 I
3723 Drd I
3788 BssS I
3821 BsaW I
3929 Alw44I
3929 ApaL I
3934 EcoRD2
3947 EcoRD2
3968 BsaW I
4031 AlwN I
4163 BspKT5I
4163 Eco57 I
4335 BspH I
4420 Rrh4273I
4421 Sal I

Transient transfection protocol for Cos-1 cells

Note: The protocol described below has been optimized for Cos-1 cells. Successful transfection of each cell type requires optimization of the basic protocol. Variables to consider for optimization include, but are not limited to: cell density, transfection reagent , duration of transfection and DNA concentration.

1. Seed a six-well tissue culture plate with 1-2 x 10^5 Cos-1 cells in 2ml DMEM supplemented with 10% fetal calf serum. Incubate for 18-24 hours at 37ºC or until the cells are 40-60% confluent in a CO_2 humidified incubator .

2. Dilute 1-2µg of DNA into 100µl serum free DMEM in a sterile tube. In a second tube dilute 20µl of LIPOFECTIN® Reagent (Life Technologies) into 100µl serum free DMEM. Allow to stand at room temperature for 30-45 minutes.

3. Combine the two solutions, mixing gently and incubate at room temperature for 10-15 minutes.

4. Wash cells once with 2ml serum free DMEM.

5. For each transfection, add 0.8ml serum-free medium to each tube containing the LIPOFECTIN® Reagent DNA complexes. Mix gently and overlay the complexes onto cells.

6. Incubate the cells for 5 hours at 37ºC in a CO_2 humidified incubator.

7. Remove the DNA-containing medium and replace with 2ml of DMEM supplemented with 10% fetal calf serum. Incubate cells 37ºC in a CO_2 humidified incubator for an additional 48-72 hours.

8. Assay cell extracts for gene expression or activity at 48-72 hours post transfection.